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Sent: Wednesday, May 14, 2003 3:05 PM
To: STIC-ILL
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Art Unit 1642 Location 8E12(mail)

Telephone Number 308-8362

Application Number 09/230,955

1. American Journal of Pathology:
   1993 Feb, 142(2):403-412
   1993, 143(4):1150-1158
   1984, 114(3):454-460
   1996, 148(3):865-875
   1965 Sep, Vol. 44, pp. 280-282

2. Cancer Research, 1993 May 15, 53(10 suppl):2287-2299

3. Cancer epidemiology, biomarkers and Prevention, 1996 Jul, 5(7):549-557

4. Lab Investigation:
   ~~1980, 42(1):91-96~~
   1988, 58(2):141-149

5. Gynecol Oncol, 1982, 13(1):58-66

6. International Journal of Gynecological Pathology:
   1985, 4(4):300-313
   1986, 5(2):151-162
   1992, 11(1):24-29

7. Differentiation:
   1986, 31(3):191-205
   1988, 39(3):185-196

8. Cancer (Phila), 1989, 63(7):1337-1342

9. Cancer Res, 1990, 50(16):5143-5152

10. Virchows Arch B Cell Pathol Incl Mol Pathol, 1987, 54 (2):98-110

11. Acta Histochemica et Cytochemica:
    1994, 27(3):251-257
    1996, 29(1):51-56

12. Archives of Gynecology and Obstetrics, 1989, 246(4):233-242

13. Clin Lab Med, 1995 Sep, 15(3):727-742

14. Clin Obstet Gynaecol, 1984 Apr, 11 (1):5-23

0023-6837/88/5802-0141$02.00/0
LABORATORY INVESTIGATION

Vol. 58, No. 2, p. 141, 1988
Printed in U.S.A.

Immunophenotypic Analysis of the Transformation Zone of Human Cervix

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The immunocompetent cell population of the cervical trasformation zone of 18 uteri removed for noncervical disease, has been investigated with monoclonal antibodies. The panel included Leu 2a, 3a, 4, 14, and IL II receptor for lymphocytes and T cell subsets, Leu 7 for NK cells, Leu M5, Leu 10, HLA-DR, DRC 1 for dendritic cells, and Leu 6 for Langerhans' cells (LC). In ectocervical epithelium HLA-DR, Leu 6 and Leu 10 antibodies identified subpopulations of dendritic cells which differed in number and in topographic distribution. Furthermore, a strong HLA-DR epithelial positivity was constantly observed in endocervical columnar cells as well as in keratinocytes of squamous metaplasia. Leu 2a+ cells (T suppressor/cytotoxic) prevailed in the stromal and epithelial compartments of ecto/endocervix; in 6 cases, however, Leu 3a+ cells (T helper/inducer) represented the main T cell subset in the ectocervical stroma. B lymphocytes were occasionally noticed in the subepithelial stroma while NK and DRC-1 cells were never observed. Finally, only few lymphocytes displayed a positivity for IL II receptor. This study suggests that several phenotypes of intraepithelial dendritic cells are present in the transformation zone and that endocervical columnar cells and keratinocytes of squamous metaplasia express HLA-DR products; the latter finding may be related to the presence of intraepithelial and stromal T lymphocytes.

Additional key words: Cervical transformation zone, Dendritic cells, Langerhans' cells, Epithelial HLA-DR expression, Antigen presenting cells, T cell subsets, Monoclonal antibodies.

Cervical transformation zone (TZ) is the site of occurrence of viral infections and related preneoplastic or neoplastic conditions (15, 62). In general, there is increasing evidence that the development and the outcome of these pathologic events is affected by the complex relationship between offending agents and the local cellular immune response (6, 8, 37, 53, 62, 66). This latter, in turn depends on the strict cooperation between lymphocyte subsets and dendritic macrophages with antigen presenting functions (9, 10, 70). A current method of investigation of these immunologic events is based on the *in situ* identification of immunocompetent cells on normal and pathologic tissue even if a well defined immunologic function cannot always be derived from immunophenotypized cells (35). Existing immunopathologic studies of the TZ have been limited in the spectrum and specificity of antibodies employed and have been focused upon specific cell types rather than composite immunophenotypic profiles of dendritic and nondendritic mononuclear cells (34, 38, 39, 46, 48, 67, 68). In fact recent studies have investigated Langerhans' (LC) and T cell populations of the TZ either in normal or in pathologic conditions, showing a reduction of LC number in papillomavirus infection (34, 39, 67). LC are epidermotropic dendritic elements involved in antigen presentation to T lymphocytes (5, 19, 55, 59, 60). In immunocompetent tissue, however, cells with supposed antigen presenting function, while showing the same dendritic morphology, display a marked topographic and phenotypic heterogeneity (19, 21, 25, 31, 32, 44, 49, 77). Furthermore, in some experimental, normal, or pathologic conditions, epithelial cells have been supposed to play an immunologic role since they express HLA-DR antigens (1–3, 30, 33, 36, 40, 47, 52, 56, 64, 65, 71, 72). These data together with the common morphologic evidence of a lympho-monocytic infiltrate in the TZ prompted us to study with a large panel of monoclonal antibodies, the immunocompetent population of this cervical site in order to characterize the lymphocytic, dendritic, and nondendritic cells of the TZ on a large homogeneous series of age-matched healthy women. This study may provide an insight into the phenotypes of cervical immunocompetent cells.

EXPERIMENTAL DESIGN

TISSUE SAMPLES

Cervical samples were collected from 18 women who underwent hysterectomy for noncervical diseases. The age ranged from 38 to 52 years. Table 1 summarizes the

main clinicopathologic features of this series. Chronic cervicitis was always observed; in particular, in 5 cases, there was a remarkable inflammatory infiltrate of the TZ with the focal collection of nodular lymphoid aggregates containing plasma cells. In 3 cases, areas of mature squamous metaplasia were also noticed. In each case, after hysterectomy, a cervical sample of the transformation zone was immediately snap frozen in liquid nitrogen and stored at −70° C until used. For conventional immunohistochemistry, 6-μm cryostat sections were dried overnight at room temperature, subsequently fixed in absolute acetone for 10 minutes at room temperature, and air dried for 20 minutes before hydrating in phosphate-buffered saline (PBS).

TABLE 1. CLINICOPATHOLOGIC CHARACTERISTICS OF THE PATIENTS STUDIED

N	Age	Surgery	Histopathology
1	41	TAH	Severe chronic cervicitis, squamous cervical metaplasia, multiple leiomyomas, proliferative endometrium
2	42	TAH	Mild chronic cervicitis, adenomyosis, secretory endometrium
3	49	TAH, BSO	Mild chronic cervicitis, adenomyosis, proliferative endometrium
4	52	TAH, BSO	Mild chronic cervicitis, leiomyoma, secretory endometrium, bilateral hydrosalpinx, bilateral ovarian follicular cysts
5	45	TAH, BSO	Severe chronic cervicitis, multiple leiomyomas, secretory endometrium, left ovarian fibrothecoma
6	38	TAH	Mild chronic cervicitis, leiomyoma, secretory endometrium
7	47	TAH, BSO	Severe chronic cervicitis, squamous cervical metaplasia, adenomyosis, secretory endometrium, luteinized ovarian cysts
8	45	TAH	Mild chronic cervicitis, multiple leiomyomas, proliferative endometrium
9	42	TAH	Mild chronic cervicitis, multiple leiomyomas, secretory endometrium
10	48	TAH, BSO	Mild chronic cervicitis, multiple leiomyomas, secretory endometrium, left ovarian follicular cyst
11	38	TAH, RSO	Mild chronic cervicitis, uterine leiomyoma, secretory endometrium, right ovarian follicular cysts
12	47	TAH, BSO	Mild chronic cervicitis, multiple leiomyomas, proliferative endometrium, bilateral ovarian follicular cysts
13	42	TAH, LSO	Mild chronic cervicitis, multiple leiomyomas, secretory endometrium
14	42	TAH	Mild chronic cervicitis, multiple leiomyomas, proliferative endometrium
15	48	TAH, BSO	Severe chronic cervicitis, multiple leiomyomas, proliferative endometrium, bilateral ovarian follicular cysts
16	43	TAH	Mild chronic cervicitis, leiomyoma, secretory endometrium
17	39	TAH, BSO	Mild chronic cervicitis, squamous cervical metaplasia, adenomyosis, proliferative endometrium, bilateral ovarian endometriosis
18	52	TAH, BSO	Severe chronic cervicitis, multiple leiomyomas, proliferative endometrium, bilateral ovarian follicular cysts

TAH, total abdominal hysterectomy; BSO, bilateral salpingoophorectomy; RSO, right salpingoophorectomy; LSO, left salpingoophorectomy.

IMMUNOCYTOCHEMISTRY

The monoclonal antibodies were used at the dilutions listed in Table 2. For Leu 2a, Leu 3a, Leu 4, and HLA-DR, the T cell panel for immunopathology (Becton-Dickinson, Mountain View, California) was employed, containing the primary antisera as well as the labeling reagents. All the sections were stained with the three stage monoclonal antibody avidin-biotin complex technique (76). Briefly, rehydrated sections were incubated with monoclonal antibody for 20 minutes. As second and third step labeling, the Vectastain Kit (Vector Laboratories, Burlingame, California) was employed for all but the T cell panel monoclonal antibodies. In between each step, sections were washed thoroughly in three changes of modified PBS (pH 7.4) (76). The reaction product was visualized by 3,3′-diaminobenzidine. Negative controls were obtained by omitting primary antisera, replaced by PBS; for evaluation of endogenous peroxidase activity, negative controls consisted of the use of chromogen alone.

QUANTITATION

Dendritic cells were identified by the presence of cytoplasmic processes (69) and were easily differentiated from round membrane-positive cells. Enumeration of positive cells was performed for the different phenotypes of dendritic cells (Leu 6+, Leu 10+, HLA-DR+, Leu M5+), either in the ectocervical basal and suprabasal epithelial layers or in subepithelial stroma. Total epithelial and subepithelial T lymphocytes (Leu 4) and lymphocyte subsets (Leu 2a, Leu 3a) were also evaluated.

Quantitation was performed in a controlled and reproducible way. Briefly, in each case, 3 to 6 pictures have

TABLE 2. MONOCLONAL ANTIBODIES

Antibody	Dilution	Predominant specificity
Anti-Leu 2a	Undiluted	Cytotoxic/suppressor T cells.
Anti-Leu 3a	Undiluted	Helper/inducer T cells, histiocytes (weakly).
Anti-Leu 4	Undiluted	T cells.
Anti-Leu 6	1:10	Langerhans' cells.
Anti-Leu 7	1:10	Large granular lymphocytes including NK cells.
Anti-Leu 10	1:10	Some (but not all) tissue macrophages, dendritic cells, B cells
Anti-Leu 14	Undiluted	B cells.
Anti-HLA-DR	Undiluted	Macrophages, dendritic cells, B cells, activated T cells, endothelial cells.
Anti-Leu M5	Undiluted	Monocytes, mature macrophages, dendritic cells.
Anti-DRC-1	1:10	B-zone dendritic reticular cells.
Anti-IL-11	1:10	Activated and proliferating T cells.

Sources: Anti-DRC-1 from Dako (Dakopatts, Santa Barbara, California). The remaining antibodies from Becton-Dickinson (Mountain View, California).

been taken under ×20 objective to document a representative area of the TZ on which the cell count was performed using a square grid of 6 cm^2. This area measured on the section 0.12 mm for each side. The mean value of positive cells counted in 6 grided areas was calculated for each case and finally expressed as number of cells/mm^2.

The internal consistency of quantitation technique was assessed for T lymphocytes in three compartments (ectocervical epithelium, ectocervical stroma, and endocervical stroma). The values of every case generated for each antibody (Leu 2a, Leu 3a, and Leu 4) were averaged to give the mean value for the compartment as a whole. Histiocytes which were variably Leu 3a+ were excluded from Leu 3a+ cell counts. The internal consistency was assessed by comparing the sum of Leu 2a+ and Leu 3a+ *versus* Leu 4+ cells in all three tissue compartments. The values in the ectocervical epithelium, ectocervical stroma, and endocervical stroma were 247 + 161 *versus* 383, 446 + 460 *versus* 929, and 505 + 270 *versus* 705, respectively.

RESULTS AND DISCUSSION

HLA-DR. In ectocervical mucosa, epithelial and stromal dendritic positive cells were constantly observed (Fig. 1*A*). Scattered subcolumnar stromal dendritic HLA-DR positive cells were also present, whereas round HLA-DR-positive mononuclear cells were only focally observed in ectocervical epithelium as well as in the stroma (Fig. 1*B*). Epithelial keratinocytes never expressed DR products with the exception of all three cases of squamous metaplasia, where groups of HLA-DR-positive keratinocytes were observed (Fig. 1*C*). Columnar cells always expressed a cytoplasmic and membranous HLA-DR positivity, strongly evident on surface epithelium and in glandular clefts (Fig. 1*D* and *E*). The membranous HLA-DR expression was seen in all aspects of plasma membranes (Fig. 1*E* and *F*). Endothelial cells of vessels were also HLA-DR-positive (Fig. 1*A*).

Leu 10. Dendritic Leu 10-positive cells were always found in ectocervical epithelium and in the stroma of the TZ. Intraepithelial Leu 10 dendritic positivity was observed in either the basal and the suprabasal cell layers (Fig. 1*G*).

Leu 6. Dendritic Leu 6-positive cells were observed in all cases only in ectocervical epithelium (Fig. 1*H*).

Leu M5. Round and dendritic positive stromal cells were focally identified, mainly in deep perivascular and periglandular stroma (Fig. 2). No Leu M5 intraepithelial positive cells were found.

Leu 2a, Leu 3a, and Leu 4. Positive staining of cell membranes of T lymphocytes (Leu 4) was observed in all specimens in cervical epithelium as well as in the stroma of the TZ (Fig. 2*A* and *B*). Scattered T lymphocytes were always found within the ectocervical epithelium, with the prevalence of the Leu 2a type (Fig. 2*C* and *D*). Intraepithelial endocervical T lymphocytes were also observed with a mild prevalence of the Leu 2a phenotype which constituted 60 to 70% of intraepithelial T lymphocytes (Fig. 2*E*). T cells subsets were mostly distributed in the subepithelial stroma (Fig. 2*F* and *G*) and sometimes clustered in nodular aggregates where Leu 3a+ cells predominated. In the TZ, some Leu 3a-positive cells with dendritic morphology were also identified.

Leu 14. Leu 14 positive cells were present only in the subepithelial stroma of the TZ, expecially in the cases showing severe chronic cervicitis.

IL II receptor. Scattered IL II receptor positive lymphocytes with a membranous pattern of staining were appreciated in the subepithelial stroma (Fig. 2*H*). These IL II receptor positive cells were frequently observed in the cases of severe chronic cervicitis.

DRC-1 and Leu 7. We did not observe any positive cell in the TZ when these monoclonal antibodies were tested.

CONTROLS

Controls, which consisted of the omission of the primary antisera, were invariably negative with the exception of the endoluminal content of some vessels, and of mast cells. In fact, mast cells sometimes presented a strong cytoplasmic granular reaction with avidin-biotin complex, which was easy to identify (Fig. 3*A*). When the chromogen alone was employed, the endogeneous peroxidase was revealed inside some vessels (Fig. 3*B*) and in the cytoplasm of granulocytes. Surface and glandular epithelium of the TZ resulted invariably negative (Fig. 3*A* and 3*B*). In addition, the aspecific staining was observed only in the above-mentioned cells and in vessels of the sections under study.

QUANTITATION

The comparative quantitative distribution of the HLA-DR+, Leu10+, Leu 6+, and Leu M5+ dendritic cells in the TZ is summarized in Figure 4. Dendritic cells in the basal epithelium showed a prevalence of the Leu 10 phenotype, whereas HLA-DR-positive cells were prevalent in either the suprabasal layer and the stromal compartment.

T Cell Subsets. In the ectocervical epithelium and in subcolumnar stroma, the T helper/suppressor ratio (± SE) was always less than 1, the mean values being 0.63 ± 0.06 and 0.61 ± 0.11, respectively. In ectocervical stroma, the T helper/suppressor ratio was more than 1 in 6 cases (mean 2.23 ± 0.39) and less than 1 in the remaining 12 cases (mean 0.86 ± 0.28).

DISCUSSION

Previous studies investigating the immunocompetent cell population of the TZ have focused the attention on LC (16, 34, 38, 48, 67). In our study, the phenotypic analysis of cells with dendritic morphology demonstrates that LC represent only one type of a larger and more complex dendritic cell population which expresses phenotypic and topographic heterogeneity. Quantitative study indicates that most of the dendritic cells are HLA-DR+ while Leu 10+ dendritic cells represent a smaller population. Anti-Leu 10 recognizes an Ia determinant, HLA-DC/DS, distinct from HLA-DR, which has been correlated with an enhanced antigen presenting capability (22). Alternatively, Most, Knapp, and Wick (40) have suggested that HLA-DC/DS can act as immune suppression gene, possibly by activating T suppressor cells. This

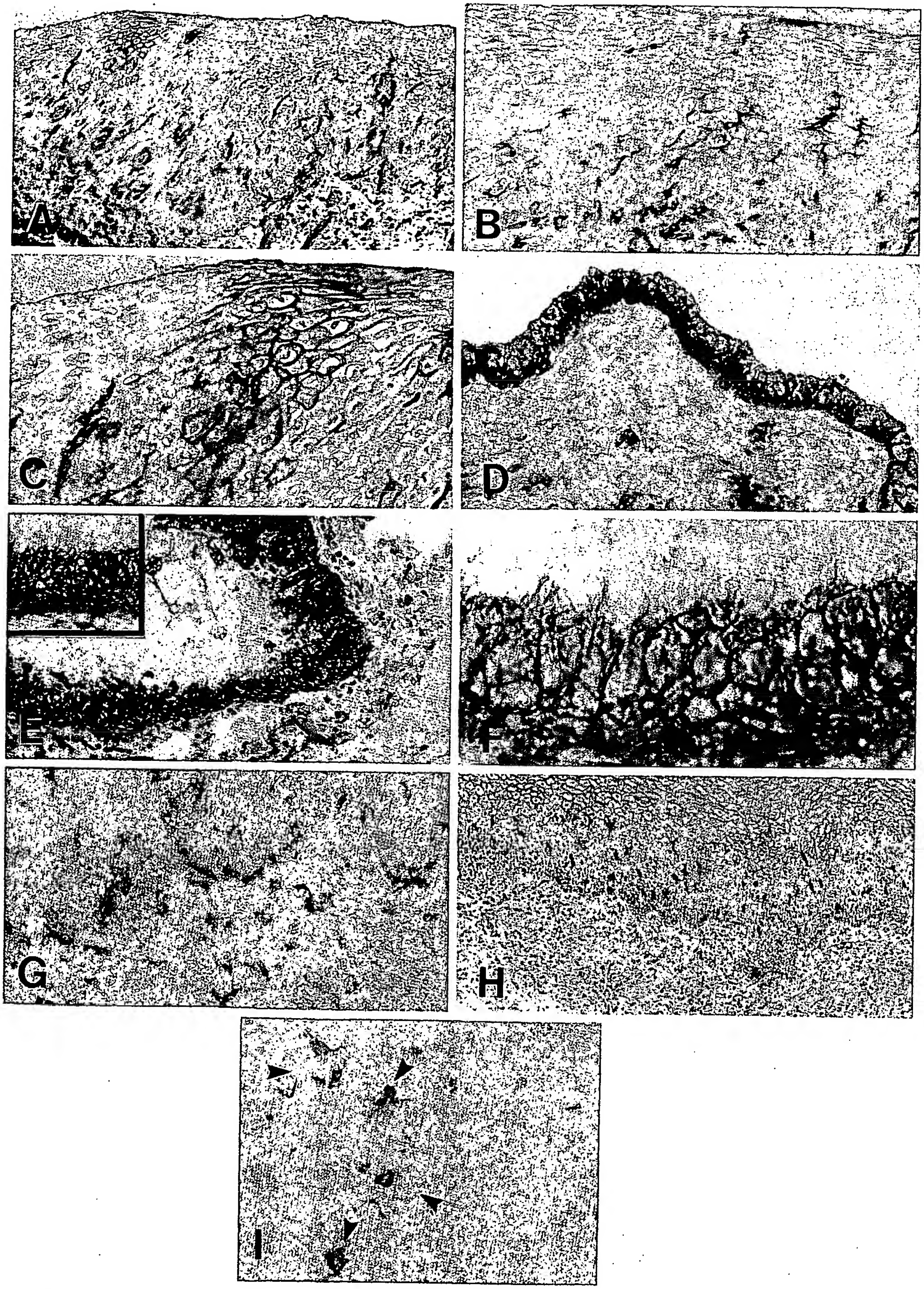

FIG. 1. Pattern of HLA-DR, Leu 10, Leu 6, and Leu M5 positive cells. *A* to *F*, HLA-DR. *A*, Dendritic intraepithelial and stromal positive cells are clearly evident, (*bottom left*), a glandular endocervical cleft lined by HLA-DR+ columnar cells and some HLA-DR+ vessels (×100, nuclear counterstain). *B*, Dendritic and round positive cells in ectocervical mucosa (×250, nuclear counterstain). *C*, An area of squamous metaplasia with keratinocytes showing marginally located cytoplasmic and surface HLA-DR positivity (×250, nuclear counterstain). *D*, Sur-

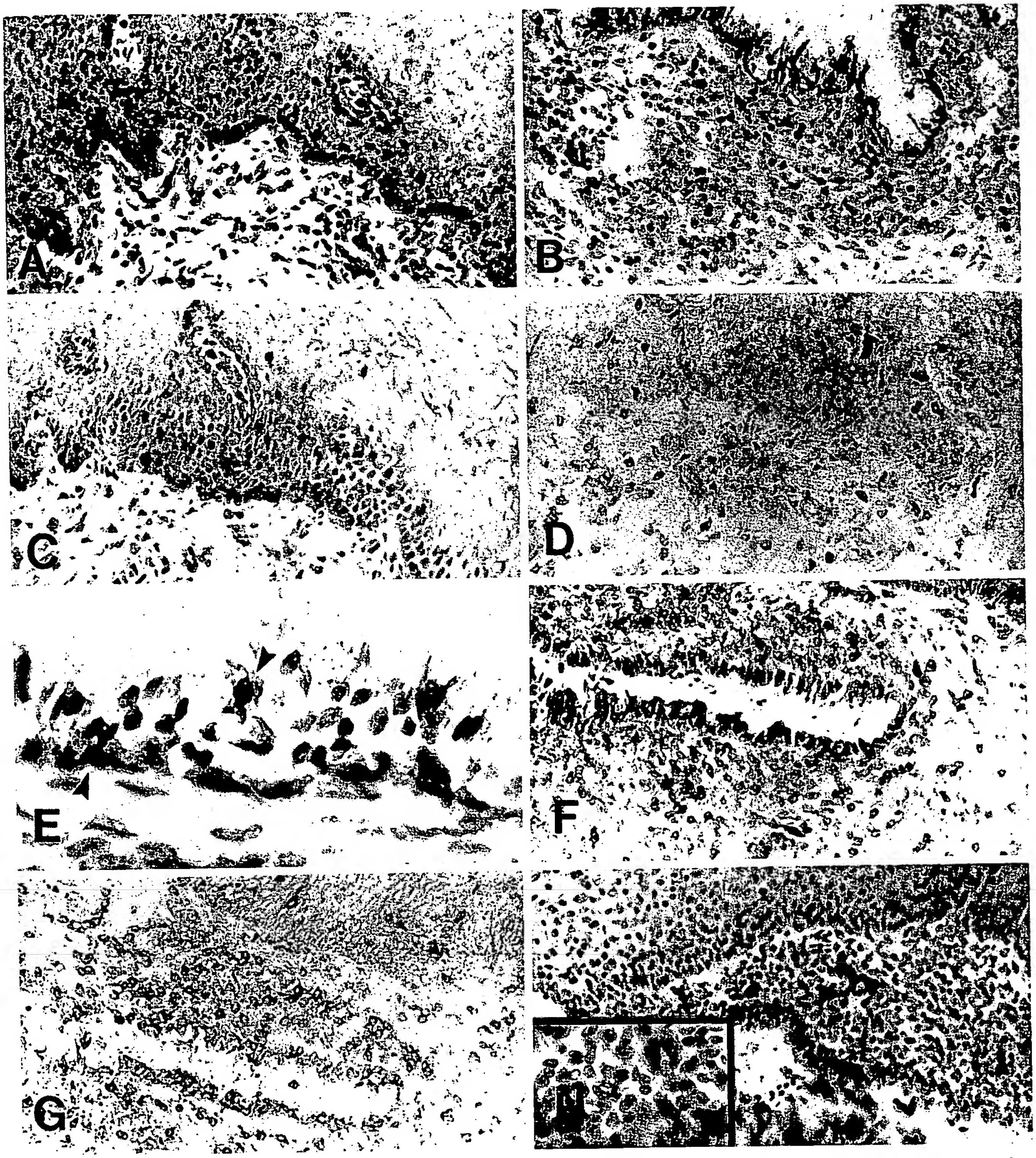

FIG. 2. Pattern of Leu 2a, Leu 3a, Leu 4 and IL II receptor positive lymphocytes. *A* and *B*, Leu 4. T lymphocytes are clearly evident either in ectocervical mucosa (A, × 250, nuclear counterstain) or in endocervical stroma (*B*, × 250, nuclear counterstain). *C* to *G*, Leu 2a and Leu 3a. *C*, Intraepithelial T lymphocytes are mainly of the Leu 2a type (×250, nuclear counterstain) as demonstrated by the comparison with *D*, showing the same field stained with Leu 3a (×250, not counterstained). *E*, Intraepithelial Leu 2a+ cells (*arrows*) in the endocervical mucosa (×400, nuclear counterstain). *F* and *G*, Same field of endocervical stroma respectively stained with Leu 2a and Leu 3a: stromal T lymphocytes are either of the T helper or T suppressor type (×250, Leu 2a with nuclear counterstain; Leu 3a, not counterstained). *H*, IL II receptor. Scattered positive stromal lymphocytes (×250, nuclear counterstain). *Inset*, Note the characteristic IL II receptor membranous pattern of positivity (×400).

face HLA-DR positive columnar cells (×300, nuclear counterstain). *E*, The same pattern of positivity of surface endocervical cells is observed in columnar cleft epithelium (×250, nuclear counterstain). *Inset*: Cytoplasmic and membranous HLA-DR positivity (×400). *F*, At higher magnification, HLA-DR details the cytoplasmic membrane of columnar cells (×1,000). *G*, Leu 10. Intraepithelial and stromal dendritic-positive cells. Note positive dendritic intraepithelial cells rimming the basal cell layer (×250, not counterstained). *H*, Leu 6. Langerhans' cells in the suprabasal ectocervical epithelium. No Leu 6-positive cells are present in the stroma (×100, nuclear counterstain). *I*, Leu M5. Leu M5 reveals few macrophages (*arrows*) in the subepithelial stroma (×400, not counterstained).

latter hypothesis fits well our observation of T suppressor/cytotoxic cells as the main lymphocytic population in the ectocervical epithelium of the TZ.

A third dendritic cell population, corresponding to LC, expresses the Leu-6 phenotype (17, 41–43). LC are identified by the ultrastructural evidence of the characteristic Birbeck's granule (4). In cervical tissue, previous investigators employed a spectrum of different antisera with variable specificity to identify LC (34, 38, 39, 48, 67). However, at present, LC are commonly immunohistochemically characterized by the Leu 6+/HLA-DR+ phenotype (11, 20, 27, 51, 73) and we have referred to this phenotype to define LC. Our quantitative results are in agreement with the study of Morris *et al.* (38), who found, on a smaller series, that the average number of LC/mm^2 varied from 74 to 145. However, we did not observe "hugging" cells located across the basement membrane. In fact, we found that LC are only present within the epithelium and, in particular, in the suprabasal layers. Our quantitative analysis indicates that, in suprabasal epithelial layers, HLA-DR+ dendritic cells outnumber Leu 6+ cells. We do not know the exact nature of these cells, but relying on their dendritic features, and their immunophenotypes (HLA-DR+/Leu 10−/M5−/ Leu 6−) we can speculate that these cells belong to the group of dendritic antigen presenting cells (21, 29, 32, 77). Finally, scattered dendritic Leu M5+ cells were distributed in the subepithelial stroma of ecto-endocervix. These cells are a minor population, which probably represents mature resting macrophages. Our study suggest the presence of different phenotypes of dendritic intraepithelial cells in the TZ. The phenotype of these dendritic cells is consistent with an antigen presenting function; however, a clear distinction among these cells, based only on immunohistochemical staining is not feasible and further studies are needed to clarify this issue.

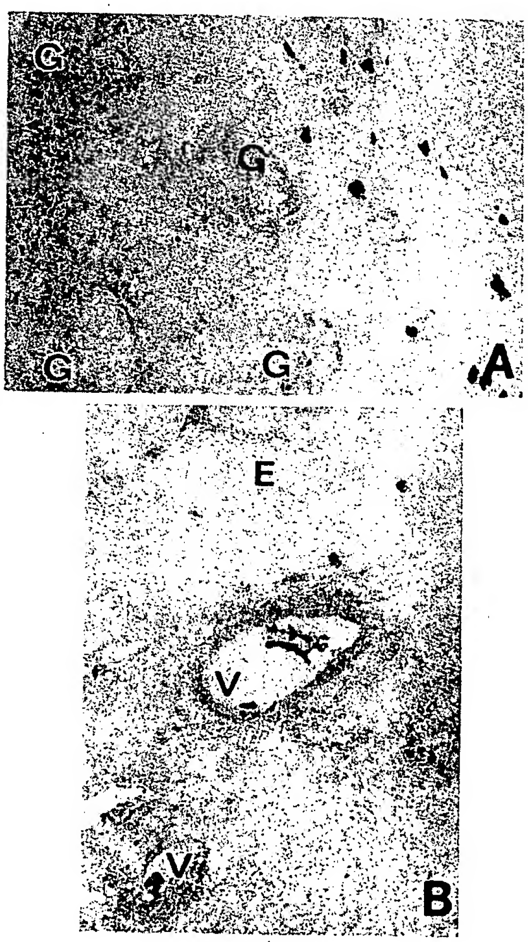

FIG. 3. Control cases. *A*, When primary antiserum was replaced by PBS, only some mast cells stained with ABC. Notice the unstained glandular clefts (*G*) (×100, nuclear counterstain). *B*, When the chromogen alone was employed, the endogenous peroxidase was revealed inside some vessels (*V*). Notice the unstained ectocervical epithelium. (×250, not counterstained).

Ia-like antigen expression by epithelial cells has been demonstrated in a wide variety of normal and abnormal conditions (2, 3, 18, 23, 26, 33, 36, 45, 56, 61, 64, 75). In particular in the cervix Morris *et al.* (38) and Puts *et al.* (48) have reported a focal HLA-DR positivity of endocervical cells. In our study, a strong epithelial HLA-DR positivity was constantly observed in columnar cells. Since chronic cervicitis with intraepithelial T lymphocytes was present in all the cases, this epithelial HLA-DR expression may be related to the abundance of T cells. In fact, in some experimental and pathologic conditions, the HLA-DR expression is induced by activated T lymphocytes as clearly demonstrated in autoimmune thyroid disease (7). The remarkable inflammatory infiltrate observed in our cases, near areas of squamous metaplasia with HLA-DR+ keratinocytes is in further support of this suggestion. In addition, a strong epithelial HLA-DR expression has been recently reported in human endometrium adjacent to lymphoid aggregates or areas of chronic endometritis by Tabibzadeh *et al.* (64). Furthermore, the same authors correlated the epithelial HLA-DR expression with the hormonal cycle, whereas in our study, the endocervical HLA-DR positivity was similarly expressed either in proliferative or in secretory phases. This is probably because the cervical epithelium does not undergo the sequences of proliferation-differentiation-secretion and shedding as seen in endometrium.

As to the immunologic significance of HLA-DR positivity on epithelial cells, Unanue and Allen (71) speculate that this antigenic expression may be related to an antigen presenting capability, although other immunologic or nonimmunologic roles cannot be excluded. According to this hypothesis, an antigen presenting capability of endocervical columnar cells is consistent with the lack of endocervical dendritic cells which, in turn, are well represented in the pluristratified squamous counterpart of the TZ.

The study of lymphocyte populations in the TZ shows that T lymphocytes greatly outnumber B lymphocytes. This finding is in keeping with the widespread presence of antigen presenting cells, which are thought to have T lymphocytes as targets (10, 50, 70, 74). As reported by other authors (38, 46, 68), T suppressor/cytotoxic cells are the main lymphocyte subset in either epithelial or stromal compartments. In particular, in ectocervical epithelium Ts cells are in close relationship with both LC

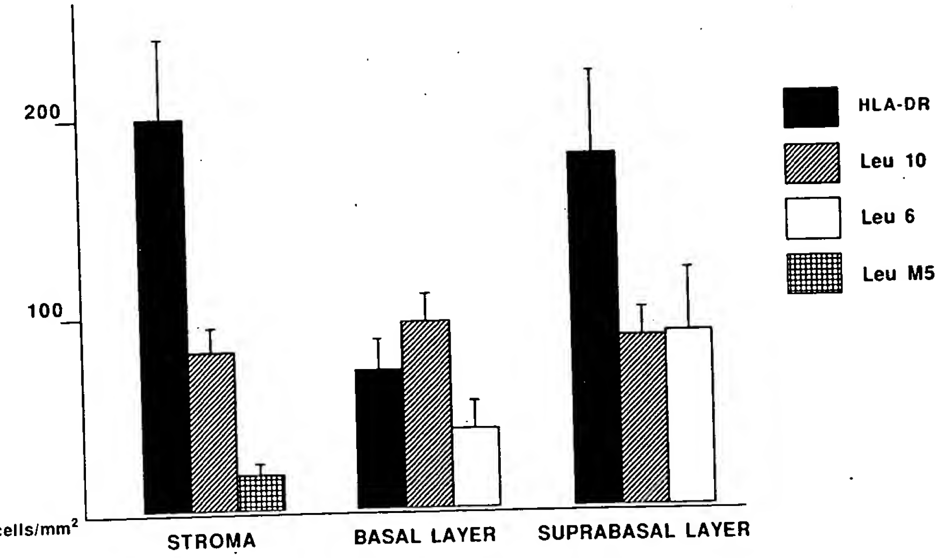

FIG. 4. Quantitative topographic comparison of HLA-DR, Leu 10, Leu 6, and Leu M5 positive dendritic cells in the epithelium and subepithelial stroma of the ectocervix. The figure represents the general averaged values of the different phenotypes of dendritic cells (mean ± SE).

and HLA-DR+ dendritic cells. This finding is in keeping with the demonstration that LC are required for the generation of cytotoxic T lymphocytes (11, 13, 59); furthermore, the response of cytotoxic T lymphocytes to allogenic targets is greatly enhanced when a class II major histocompatibility antigenic stimulus is also provided (14, 28, 54, 57, 58). In the subepithelial ectocervical stroma, however, one-third of the cases showed a prevalence of Leu 3a+ cells, especially in the cases showing B lymphocytes and plasma cells. The latter observation, as suggested by Morris *et al.* (38), may be correlated with the known role of T helper/inducer cells in differentiating B lymphocytes into plasma cells. IL II receptor positivity, indicative of functionally active and proliferating lymphocytes (24), was focally expressed, especially in cases of severe chronic cervicitis. This finding confirms the resting condition of most of the lymphocytes present in the human adult cervical mucosa, and correlates well with the almost total absence of NK cells. This latter observation, however, is in disagreement with that reported by Syrjanen *et al.* (63), who found scattered NK cells in the cervical TZ. The clinical characteristics of the population studied by these authors, mainly affected by HPV infection, may account for these discrepancies.

Acknowledgments: The Authors are greatly indebted to Mrs. Olivia Senesi for the skillful technical assistance. We also thank Dr. Renato Gori (Becton Dickinson Italia, Società per Azioni) for the technical collaboration.

Date of acceptance: September 18, 1987.
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REFERENCES

1. Arnaud-Battandier F, Cerf-Bensussan N, Amsellem R, Schmitz J: Increased HLA-DR expression by enterocytes in children with celiac disease. Gastroenterology 91:1206, 1986
2. Aubock J, Romani N, Grubauer G, Fritsch P: HLA-DR expression on keratinocytes is a common feature of diseased skin. Br J Dermatol 114:465, 1986
3. Bernard DJ, Maurizis JC, Chassagne J, Chollet P, Plagne R: Comparison of class II HLA antigen expression in normal and carcinomatous human breast cells. Cancer Res 45:1152, 1985
4. Birbeck MS, Breathnach AS, Everall JD: An electron microscopic study of basal melanocytes and high level clear cells (Langerhans' cell) in vitiligo. J Invest Dermatol 37:51, 1961
5. Braathen LR, Thorsby E: Studies on human epidermal Langerhans' cells. I. Allo-activating and antigen-presenting capacity. Scand J Immunol 11:401, 1980
6. Breathnach SM, Katz SI: Cell-mediated immunity in cutaneous disease. Human Pathol 17:161, 1986
7. Burek CL, Rose NR: Cell-mediated immunity in autoimmune thyroid disease. Human Pathol 17:246, 1986
8. Chardonnet Y, Beauve P, Viac J, Schmitt D: T-cell subsets and Langerhans' cells in wart lesions. Immunol Let 6:191, 1983
9. Claman NH, Chaperon EA, Triplett RF: Thymus-marrow cell combinations: synergism in antibody production. Proc Soc Exp Biol Med 122:1167, 1966
10. Cramer DV, Gill TJ III: Genetic aspects of cellular interactions in the immune response. Lab Invest 55:126, 1986
11. Czernielewski J, Faure M, Schmitt D, Thivolet J: *In vitro* mixed skin cell lymphocyte culture reaction (MSLR) in man: analysis of the epidermal cell and T cell subpopulations. Clin Exp Immunol 50:426, 1982
12. Drijkoningen M, de Wolf-Peeters C, van den Oord JJ, Degreef H, Desmet V: Expression of HLA-DR and T6 antigens on keratinocytes and dendritic cells. Arch Pathol Lab Med 110:321, 1986
13. Faure M, Frappaz A, Schmitt D, Dezutter-Dambuyant C, Thivolet J: Role of HLA-DR bearing Langerhans' cells in the *in vitro* generation of alloreactive cytotoxic T cells in man. Cell Immunol 83:271, 1984
14. Feighery C, Stastny P: HLA-D region associated determinants serve as targets for human cell-mediated lysis. J Exp Med 149:485, 1979
15. Fenoglio CM: Viruses in the pathogenesis of cervical neoplasia: an update. Human Pathol 13:785, 1982
16. Figueroa J, Caorsi I: An ultrastructural and morphometric study of Langerhans' cells in normal human ectocervix. J Anat 131:669, 1981
17. Fithian E, Kung PC, Goldstein G, Rubenfeld M, Fenoglio C, Edelson R: Reactivity of Langerhans' cells with hybridoma antibody. Proc Natl Acad Sci USA 78:2541, 1981
18. Forsum U, Klareskog L, Peterson PA: Distribution of Ia-antigen like molecules on non-lymphoid tissues. Scand J Immunol 9:343, 1979
19. Fossum S, Ford WL: The organization of cell populations within lymph nodes: their origin, life history and functional relationships. Histopathology 9:469, 1985
20. Foster CA, Holbrook KA, Farr AG: Ontogeny of Langerhans' cells in human embryonic and fetal skin: expression of HLA-DR and

OKT-6 determinants. J Invest Dermatol 86:240, 1986
21. Franklin WA, Mason DY, Pulford K, Falini B, Bliss E, Gatter KC, Stein H, Clarke LC, McGee JO'D: Immunohistological analysis of human dendritic cells by using monoclonal antibodies. Lab Invest 54:322, 1986
22. Gonwa TA, Picker LJ, Raff HV, Goyert SM, Silver J, Stobo JD: Antigen-presenting capabilities of human monocytes correlates with their expression of HLA-DS, an Ia determinant distinct from HLA-DR. J Immunol 130:706, 1983
23. Hart DNJ, Fabre JW: Endogenously produced Ia antigens within cells of convoluted tubules of rat kidney. J Immunol 126:2109, 1981
24. Jacques Y, Soulillou JP: Third French Workshop on IL-2. Joint report. Lymphokine Res 4:159, 1985
25. Jones EL: Reticulum cells: characterization and immune functions and the nature of Hodgkin and Reed-Sternberg cells. J Pathol 144:227, 1984
26. Klareskog L, Forsum U, Peterson PA: Hormonal regulation of the expression of Ia antigens on mammary gland epithelium. Eur J Immunol 10:958, 1980
27. Klareskog L, Malmnas Tjernlund U, Forsum U, Peterson PA: Epidermal Langerhans' cells express Ia antigens. Nature 268:248, 1977
28. Klein J, Chiang CL, Hauptfeld V: Histocompatibility antigens controlled by the I region of the murine H-2 complex. Cell mediated lymphocytotoxicity. J Exp Med 145:450, 1977
29. Lafuse WP, David CS: Ia antigens: genes, molecules and function. Transplantation 38:443, 1984
30. Londei M, Lamb JR, Bottazzo GF, Feldman M: Epithelial cells expressing aberrant MHC class II determinants can present antigen to cloned human T cells. Nature 312:639, 1984
31. MacPherson GG, Pugh CW: Heterogeneity amongst lymph-borne "dendritic" cells. Immunobiol 168:338, 1984
32. Marder P, Hinson A, Russo C, Ferrone S, Ades E: Heterogeneity of human peripheral blood mononuclear cells detected by monoclonal antibodies to monomorphic determinants of human Ia antigens. Immunobiol 167:483, 1984
33. Mayrhofer G, Pugh CW, Barclay AN: The distribution, ontogeny and origin in the rat of Ia-positive cells with dendritic morphology and of Ia antigen in epithelia, with special reference to the intestine. Eur J Immunol 13:112, 1983
34. McArdle JP, Muller KH: Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection. Am J Obstet Gynecol 154:509, 1986
35. McCluskey RT, Bhan AK: Cell-mediated immunity in renal disease. Human Pathol 17:146, 1986
36. McNicol AM: Class II MHC antigen expression in adrenal cortex. Lancet ii:1282, 1986
37. Mohanty KC, Roy RB: Thymus derived lymphocytes (T cells) in patients with genital warts. Br J Vener Dis 60:186, 1984
38. Morris HHB, Gatter KC, Stein H, Mason DY: Langerhans' cells in human cervical epithelium: an immunohistological study. Br J Obstet Gynecol 90:400, 1983
39. Morris HHB, Gatter KC, Sykes G, Casemore V, Mason DY: Langerhans' cells in human cervical epithelium: effects of wart virus infection and intraepithelial neoplasia. Br J Obstet Gynecol 90:412, 1983
40. Most J, Knapp W, Wick G: Class II antigens in Hashimoto thyroiditis. I. Synthesis and expression of HLA-DR and HLA-DQ by thyroid epithelial cells. Clin Immunol Immunopathol 41:165, 1986
41. Murphy GF: Monoclonal anti-T6 antibody and Langerhans' cells. Br J Dermatol 107:487, 1982
42. Murphy GF, Bhan AK, Sato S, Harrist TJ, Mihm MC Jr: Characterization of Langerhans' cells by the use of monoclonal antibodies. Lab Invest 45:465, 1981
43. Murphy GF, Bhan AK, Sato S, Mihm MC Jr, Harrist TJ: A new immunologic marker for human Langerhans' cells. New Engl J Med 304:971, 1981
44. Murphy GF, Messadi D, Fonferko E, Hancock WW: Phenotypic transformation of macrophages to Langerhans' cells in the skin. Am J Pathol 123:401, 1986
45. Parr EL, McKenzie IFC: Demonstration of Ia antigens on mouse intestinal epithelial cells by immunoferritin labeling. Immunogenetics 8:499, 1979
46. Peters WM: Nature of "basal" and "reserve" cells in oviductal and cervical epithelium in man. J Clin Pathol 39:306, 1986
47. Poulsen LO, Elling P, Brandt Sorensen F, Hoedt-Rasmussen K: HLA-DR expression and disease activity in ulcerative colitis. Scand J Gastroenterol 21:364, 1986
48. Puts JJG, Moekser O, de Waal RMW, Kenemans P, Vooijs GP, Ramaekers FCS: Immunohistochemical investigation of Langerhans' cells in normal epithelium and in epithelial lesions of the uterine cervix. Int J Gynecol Pathol 5:151, 1986
49. Radzun HJ, Parwaresch MR, Feller AC, Hansmann ML: Monocyte/macrophage-specific monoclonal antibody Ki-Ml recognizes interdigitating reticulum cells. Am J Pathol 117:441, 1984
50. Rosenthal AS: Regulation of the immune response-role of the macrophage. New Engl J Med 303:1153, 1980
51. Rowden G, Lewis MG, Sullivan AK: Ia antigen expression on human epidermal Langerhans' cells. Nature 268:247, 1977
52. Scheynius A, Johansson C, Van der Meide PH: *In vivo* induction of Ia antigens on rat keratinocytes by gamma-interferon. Br J Dermatol 115:543, 1986
53. Schneider V, Kay S, Lee HM: Immunosuppression as a high risk factor in the development of condyloma acuminatum and squamous neoplasia of the cervix. Acta Cytol 27:220, 1983
54. Schwartz RH: T-lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. Annu Rev Immunol 3:237, 1985
55. Shelley WB, Juhlin L: Langerhans' cells form a reticuloepithelial trap for external contact antigens. Nature 261:259, 1976
56. Smolle J: HLA-DR-antigen bearing keratinocytes in various dermatologic disorders. Acta Dermatol Venereol 65:9, 1985
57. Sollinger HW, Bach FH: Collaboration between *in vivo* immune responses: LS, LD, and SD antigens of major histocompatibility complex. Nature 259:487, 1976.
58. Spits H, Ijssel, Thompson A, de Vries JE: Human T4+ and T8+ cytotoxic T lymphocyte clones directed at products of different class II major histocompatibility complex loci. J Immunol 131:678, 1983
59. Stingl G, Katz SI, Clement L, Green I, Shevach EM: Immunologic functions of Ia-bearing epidermal Langerhans' cells. J Immunol 121:2005, 1978
60. Streilein JW, Bergstresser PR: Langerhans' cells: antigen presenting cells of the epidermis. Immunobiology 168:285, 1984
61. Sutton L, Mason DY, Redman CWG: HLA-DR positive cells in human placenta. Immunology 49:103, 1983
62. Syrjanen KJ: Human papillomavirus (HPV) infections of the female genital tract and their associations with intraepithelial neoplasia and squamous cell carcinoma. Pathol Annu 21:53, 1986
63. Syrjanen K, Vayrynen M, Mantyjarvi R, Castren O, Saarikoski S: Natural killer cells with HNK-1 phenotype in the cervical biopsies of women followed-up for human papillomavirus (HPV) lesions. Acta Obstet Gynecol Scand 65:139, 1986
64. Tabibzadeh SS, Bettica AM, Gerber MA: Variable expression of Ia antigens in human endometrium and in chronic endometritis. Am J Clin Pathol 86:153, 1986
65. Tabibzadeh SS, Gerber MA, Satyaswaroop PG: Induction of HLA-DR antigen expression in human endometrial epithelial cells *in vitro* by recombinant gamma-interferon. Am J Pathol 125:90, 1986
66. Tabibzadeh SS, Koss LG, Molnar J, Romney S: Association of human papillomavirus with neoplastic processes in the genital tract of four women with impaired immunity. Gynecol Oncol 12:129, 1981
67. Tay SK, Jenkins D, Maddox P, Campion M, Singer A: Subpopulation of Langerhans' cells in cervical neoplasia. Br J Obstet Gynecol 94:10, 1987
68. Tay SK, Jenkins D, Maddox P, Singer A: Lymphocyte phenotypes in cervical intraepithelial neoplasia and human papillomavirus infection. Br J Obstet Gynecol 94:16, 1987
69. Tew JG, Thorbecke J, Steinman RM: Dendritic cells in the immune response: characteristics and recommended nomenclature (a report from the Reticuloendothelial Society Committee on Nomenclature). J Reticuloendothel Soc 31:371, 1982
70. Unanue ER: Antigen-presenting function of the macrophage. Ann Rev Immunol 2:395, 1984
71. Unanue ER, Allen PM: Comment on the finding of Ia Expression in nonlymphoid cells. Lab Invest 55:123, 1986
72. Van den Oord JJ, de Vos R, Desmet VJ: *In situ* distribution of major histocompatibility complex products and viral antigens in chronic hepatitis B virus infection: evidence that HBc-containing

hepatocytes may express HLA-DR antigens. Hepatology 6:981, 1986

73. Weiss LM, Beckstead JH, Warnke RA, Wood GS: Leu-6-expressing cells in lymph nodes: dendritic cells phenotypically similar to interdigitating cells. Human Pathol 17:179, 1986
74. Werdelin O: Determinant protection: a hypothesis for the activity of immune response genes in the processing and presentation of antigens by macrophages. Scand J Immunol 24:625, 1986
75. Wiman K, Curman B, Tragardh L, Peterson PA: Demonstration of HLA-DR-like antigens on milk fat globule membranes. Eur J Immunol 9:109, 1979
76. Wood GS, Warnke RA: Suppression of endogenous avidin-binding activity in tissues and its relevance to biotin-avidin detection systems. J Histochem Cytochem 29:1196, 1981
77. Wood GS, Turner RR, Shiurba RA, Eng L, Warnke RA: Human dendritic cells and macrophages: *in situ* immunophenotypic definition of subsets that exhibit specific morphologic and microenvironmental characteristics. Am J Pathol 119:73, 1985